

CLAIMS

1. (Currently Amended) A method of cultivating cancer cells from human tissue for molecular-biological mass screenings wherein a tissue sample is locally separated into disk segments by sequential and parallel mechanical splitting based on its heterogeneous structure of tumor cells, normal cells, and contaminants, and wherein said separated tissue sample segments are further split into tissue fragments, and wherein said separated tissue fragments and fluids of the locally separated tissue sample segments are selectively cultivated in a specific culture medium for cultivating tumor cells and under predefined cultivation conditions and under suppression of the disturbing influence of normal cells and contaminants, and wherein the tissue fragments and fluids obtained from the locally separated tissue sample segments are cultivated separately in cell culture bottles filled with said culture medium for cultivating tumor cells and coated with a biomatrix substrate in an 0.01% to 3% oxygen atmosphere, an 0.1% to 5% carbon dioxide atmosphere, at a humidity of 100% and temperatures in the range from 30°C to 36.5°C and wherein said tissue sample is temporarily placed in a culture medium for storage together with adhering erythrocytes from the collection site of the sample in the respective patient until the tissue sample fragments are produced, and wherein the tissue sample is kept in the culture medium for storage for a minimum of 2 hours but no longer than 24 hours at temperature in the range from 4°C to 12°C to get adapted to said culture medium for storage, and

wherein the culture medium for cultivating tumors is composed of inorganic salts,
specifically

<u>Ca(NO₃)₂</u>	<u>10-100</u>	<u>mg/L</u>
<u>CaCl₂ · 2H₂O</u>	<u>80-150</u>	<u>mg/L</u>
<u>KCl</u>	<u>200-1000</u>	<u>mg/L</u>
<u>MgSO₄ · 7H₂O</u>	<u>200-700</u>	<u>mg/L</u>
<u>NaCl</u>	<u>3000-10000</u>	<u>mg/L</u>
<u>NaHCO₃</u>	<u>1500-4000</u>	<u>mg/L</u>
<u>Na₂HPO₄</u>	<u>100-1000</u>	<u>mg/L;</u>

amino acids, specifically

<u>L-arginine · 4 HCl</u>	<u>10-500</u>	<u>mg/L</u>
<u>L-asparagine (free base)</u>	<u>10-500</u>	<u>mg/L</u>
<u>L-aspartic acid</u>	<u>10-500</u>	<u>mg/L</u>
<u>L-cystine</u>	<u>10-500</u>	<u>mg/L</u>
<u>L-glutamic acid</u>	<u>10-500</u>	<u>mg/L</u>
<u>L-glutamine</u>	<u>10-500</u>	<u>mg/L</u>
<u>Glycine</u>	<u>10-500</u>	<u>mg/L</u>
<u>L-histidine (free base)</u>	<u>10-500</u>	<u>mg/L</u>
<u>L-hydroxyproline</u>	<u>10-500</u>	<u>mg/L</u>
<u>L-isoleucine</u>	<u>10-500</u>	<u>mg/L</u>

<u>L-leucine</u>	<u>10-500</u>	<u>mg/L</u>
<u>L-lysine · HCl</u>	<u>10-500</u>	<u>mg/L</u>
<u>L-methionine</u>	<u>10-500</u>	<u>mg/L</u>
<u>L-phenylalanine</u>	<u>10-500</u>	<u>mg/L</u>
<u>L-proline</u>	<u>10-500</u>	<u>mg/L</u>
<u>L-serine</u>	<u>10-500</u>	<u>mg/L</u>
<u>L-threonine</u>	<u>10-500</u>	<u>mg/L</u>
<u>L-tryptophane</u>	<u>5-400</u>	<u>mg/L</u>
<u>L-tyrosine</u>	<u>10-500</u>	<u>mg/L</u>
<u>L-valine</u>	<u>10-500</u>	<u>mg/L</u>
<u>L-alanine</u>	<u>10-300</u>	<u>mg/L</u>

vitamins, specifically

<u>Biotin</u>	<u>0,01-10</u>	<u>mg/L</u>
<u>D-Ca-pantothenate</u>	<u>0,01-10</u>	<u>mg/L</u>
<u>Choline chloride</u>	<u>0,1-50</u>	<u>mg/L</u>
<u>Folic acid</u>	<u>0,01-10</u>	<u>mg/L</u>
<u>i-Inositol</u>	<u>0,1-100</u>	<u>mg/L</u>
<u>Niacin amide</u>	<u>0,01-10</u>	<u>mg/L</u>
<u>Pyridoxine · HCl</u>	<u>0,01-10</u>	<u>mg/L</u>
<u>Riboflavin</u>	<u>0,1-100</u>	<u>mg/L</u>
<u>Thiamine · HCl</u>	<u>0,1-50</u>	<u>mg/L</u>

<u>Paraminobenzoic acid</u>	<u>1-1000</u>	<u>mg/L</u>
<u>Vitamin B₁₂</u>	<u>1-1000</u>	<u>mg/L</u>
<u>Niacin</u>	<u>1-100</u>	<u>mg/L</u>
<u>Ascorbic acid</u>	<u>1-5000</u>	<u>mg/L</u>
<u>Folinic acid</u>	<u>1-100</u>	<u>mg/L</u>
<u>Liponic acid</u>	<u>1-100</u>	<u>mg/L</u>
<u>Vitamin A (acetate)</u>	<u>10-1000</u>	<u>mg/L</u>
<u>Pyridoxine · HCl</u>	<u>1-100</u>	<u>mg/L</u>
<u>Niacinamide</u>	<u>1-100</u>	<u>mg/L</u>
<u>α-Tocopherol phosphate</u>	<u>0-1000</u>	<u>mg/L</u>

and

<u>D-glucose</u>	<u>100-5000</u>	<u>mg/L</u>
<u>Phenol red</u>	<u>0,1-1000</u>	<u>mg/L</u>
<u>Glutathione (reduced)</u>	<u>0,01-10</u>	<u>mg/L</u>
<u>Sodium pyruvate</u>	<u>0,1-50</u>	<u>nM</u>
<u>Epidermal growth factor (EGF)</u>	<u>1-3000</u>	<u>ng/L</u>
<u>Fetal bovine serum (FBS)</u>	<u>12.5%</u>	
<u>Bovine insulin (lyophilisate)</u>	<u>0.1-50</u>	<u>mg/L</u>

and antibiotics.

2. (Previously Presented) The method according to claim 1 wherein said tissue sample is obtained from fine needle, aspiration, intraoperative biopsies or a resection sample.
3. (Currently Amended) The method according to claim 1, wherein the culture medium for storage of the freshly taken sample and the culture medium ~~to be used~~ for cultivating ~~the~~ tumor cells are identical.
4. (Cancelled)
5. (Cancelled).
6. (Previously Presented) The method according to claim 1 wherein the medium in the culture bottle is replaced by a fresh medium of the same composition some time after initial establishment of the cell culture and completed adhesion.
7. (Original) The method according to claim 6 wherein the medium is replaced depending on the presence of contaminants such as bacteria and fungi, containing either the same or a reduced portion of antibiotics.
8. (Cancelled)

9. (Withdrawn) An apparatus for performing the method according to claim 1 to produce a locally separated preparation of a tissue sample, characterized in that it comprises a cutting apparatus for sequential and parallel splitting of the tissue sample (6) into individual tissue segments (6a) and the tissue fluid resulting from the respective cut edge, said cutting apparatus consisting of a collecting pan (1) divided into chambers and sub-chambers (1a1 through 1a10, 1b1 through 1b5, 1c1 through 1c3, 1d, and 1e1 through 1e10), a cutting plate (2) detachably mounted on top of said collecting pan (1) and having cutting grooves (4) that are partly open towards the collecting pan, a cutting blade frame (3) for cutting knives (10), the arrangement of said cutting knives (10) in the cutting blade frame (3) matching that of the cutting grooves (4) in the cutting plate (2), and that each cutting groove (4) is assigned to a single sub-chamber located beneath it; as well as a grinding unit for the further preparation of the locally separated tissue sample segments (6a) comprising a fluid-collecting pan (13) that is divided into chambers (13a through 13e), a detachably mounted preparation plate (15) with recesses (16) on top of it, and rotatory plungers (18) with plunger knives (19), said recesses (16) comprising holes (17) and being located above a chamber (13a through 13e) while said rotatory plungers (18) are associated with said recesses (16).

10. (Withdrawn) The apparatus according to claim 9, characterized in that said cutting plate (2) comprises a roughened support surface (5) in its center that runs in vertical direction to said cutting grooves (4) and keeps the tissue sample (6) in a stable position.

11. (Withdrawn) The apparatus according to claim 9, characterized in that said cutting grooves (4) that run under said support surface (5) are open towards the sub-chambers located underneath so that the tissue fluid resulting from cutting or tissue pieces are collected separately in the respective sub-chamber.

12. (Withdrawn) The apparatus according to claim 9, characterized in that the width of the cutting grooves (4) is greater than the thickness of the cutting knives (10).

13. (Withdrawn) The apparatus according to claim 9, characterized in that said cutting knives (10) are mounted to a cover plate of the cutting blade frame (3).

14. (Withdrawn) The apparatus according to claim 9, characterized in that said cutting knives (10) are designed as cutting wires that stretch inside the cutting blade frame (3).

15. (Withdrawn) The apparatus according to claim 9, characterized in that said cutting plate (2) and preparation plate (15) are each kept in guide rails (7 or 14, respectively) on said collecting pan (1) or fluid-collecting pan (13), said guide rails (7) for the cutting plate (2) having recesses (8) in alignment with said cutting grooves (4).

16. (Withdrawn) The apparatus according to claim 9, characterized in that said rotatory plungers (18) are mounted in holes of a plunger base plate (20) and can be rotated and moved up and down.

17. (Cancelled).